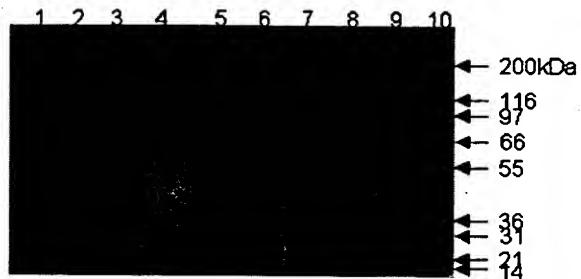
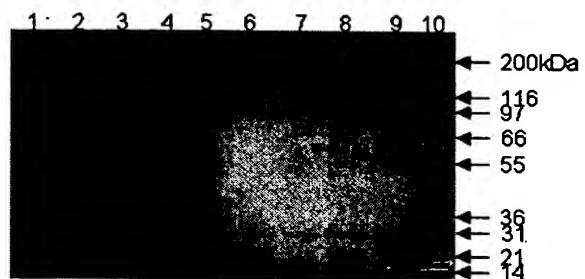


Fig. 1



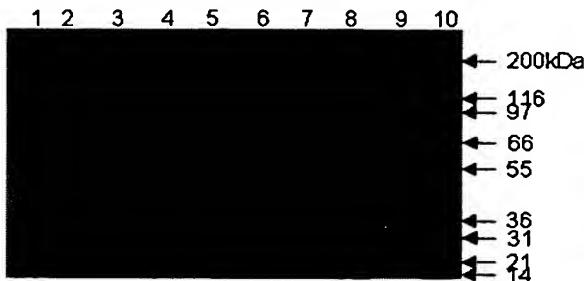
Lanes 1 & 10, marker proteins; lane 2 untreated mbh; lane 3, 50°C; lane 4, 60°C; lane 5, 70°C; lane 6, 80°C; lane 7, 90°C; lane 8, 100°C; lane 9, Protease M.

Fig. 2



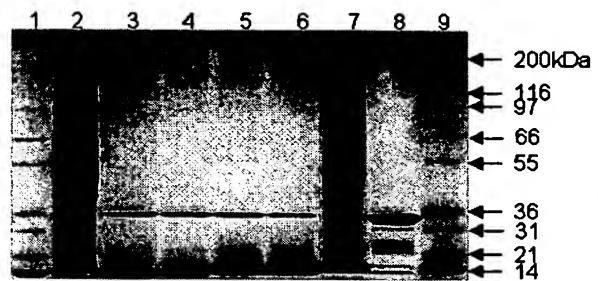
Lanes 1 & 10, marker proteins; lane 2 untreated mbh; lane 3, pH2, lane 4, pH4, lane 5, pH6; lane 6, pH8; lane 7, pH10; lane 8, pH12; lane 9, Protease M.

Fig. 3



Lanes 1 & 10, marker proteins; lane 2 untreated mbh; lanes 3 - 8, Rokko digest (20mg.ml^{-1} - 0.1mg.ml^{-1}), lane 9, Rokko (1mg.ml^{-1}).

Fig. 4



Lanes 1 & 9, marker proteins; lane 2 untreated mbh; lane 3, 2% SDS; lane 4, 1% SDS; lane 5, 0.5% SDS; lane 6, 0.25% SDS; lane 7, mbh + 2% SDS; lane 8, Rokko (20mg.ml^{-1}).

Fig. 5

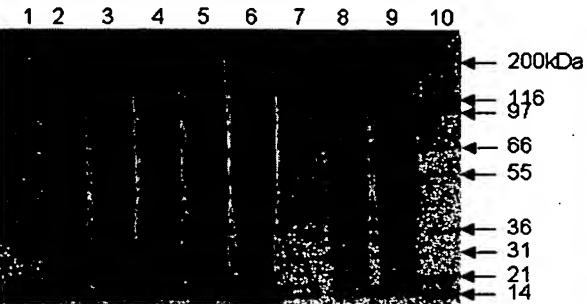
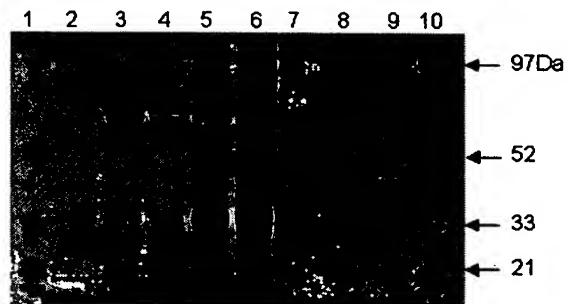


Fig. 6



Lanes 1 & 10, marker proteins; lanes 2 & 3, mbh; lanes 4 - 6, mbh pellet; lanes 7 - 9, mbh supernatant.

Fig.7

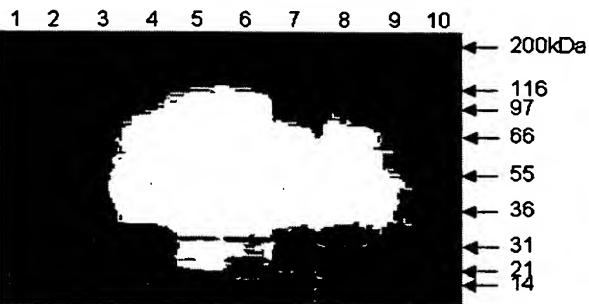
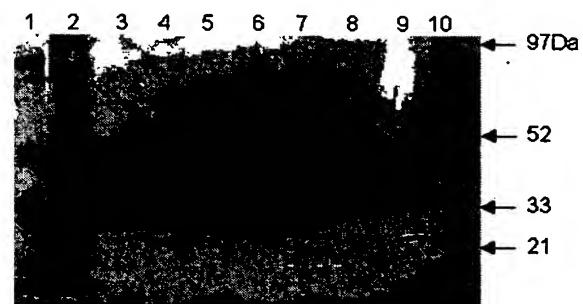


Fig.8



Lanes 1 & 10, marker proteins; lane 2, untreated mbh; lane 3, Protease G digest; lane 4, Protease G; lane 5, Protease R digest; lane 6, Protease R; lane 7, Protease C digest; lane 8, Protease C; lane 9, rec. mouse PrP.

1

2

3

4

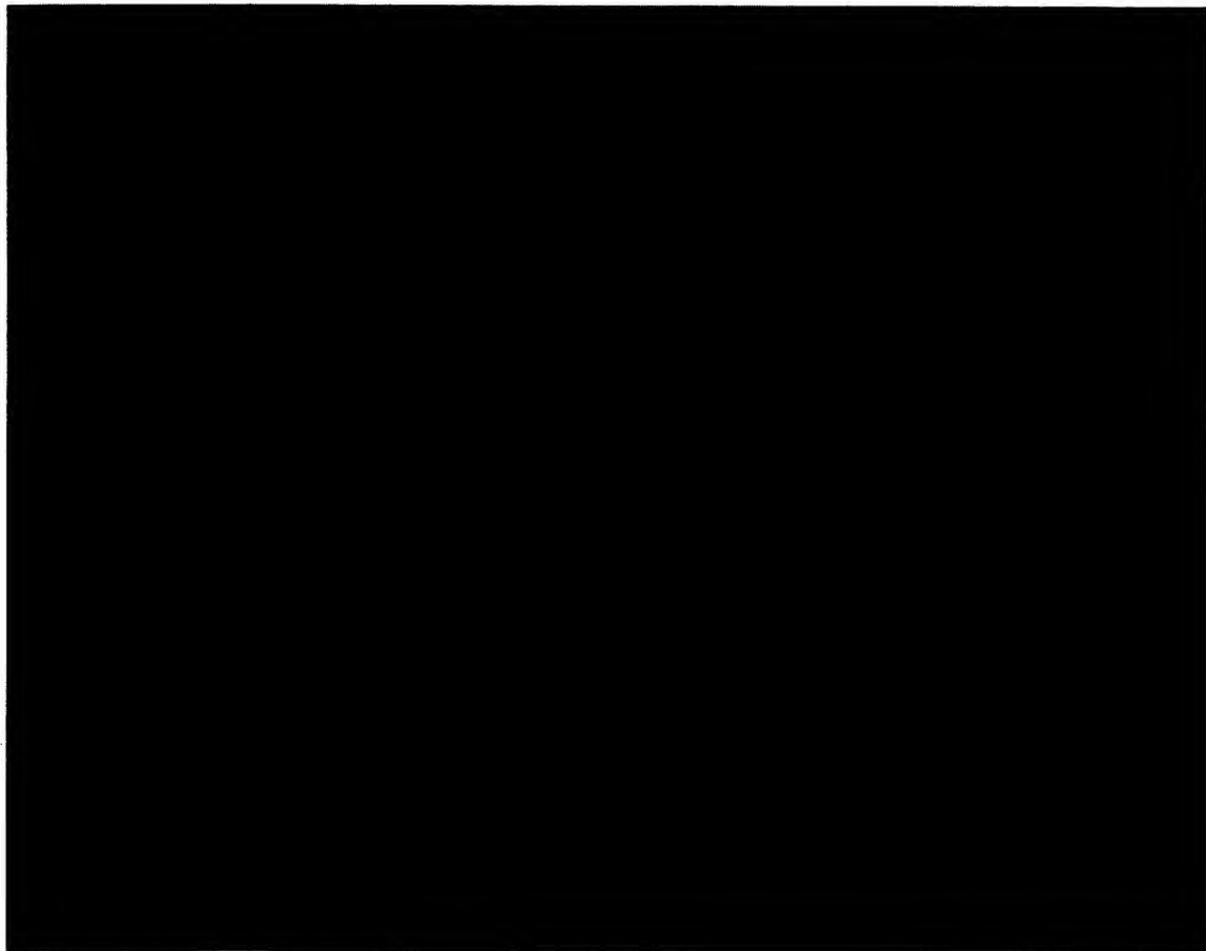


Fig. 9

5

6

7

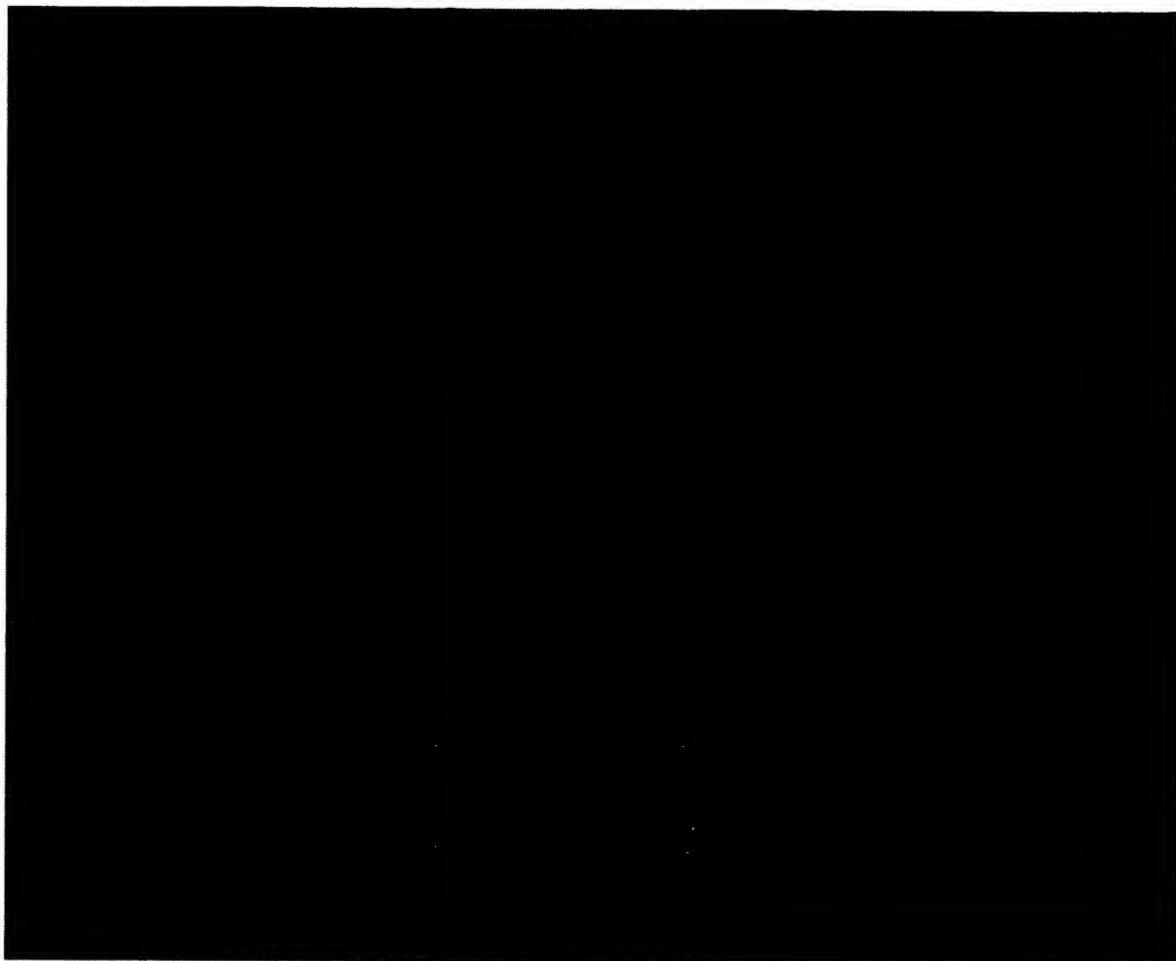


Fig. 10

1

2



Fig. 11

3

7

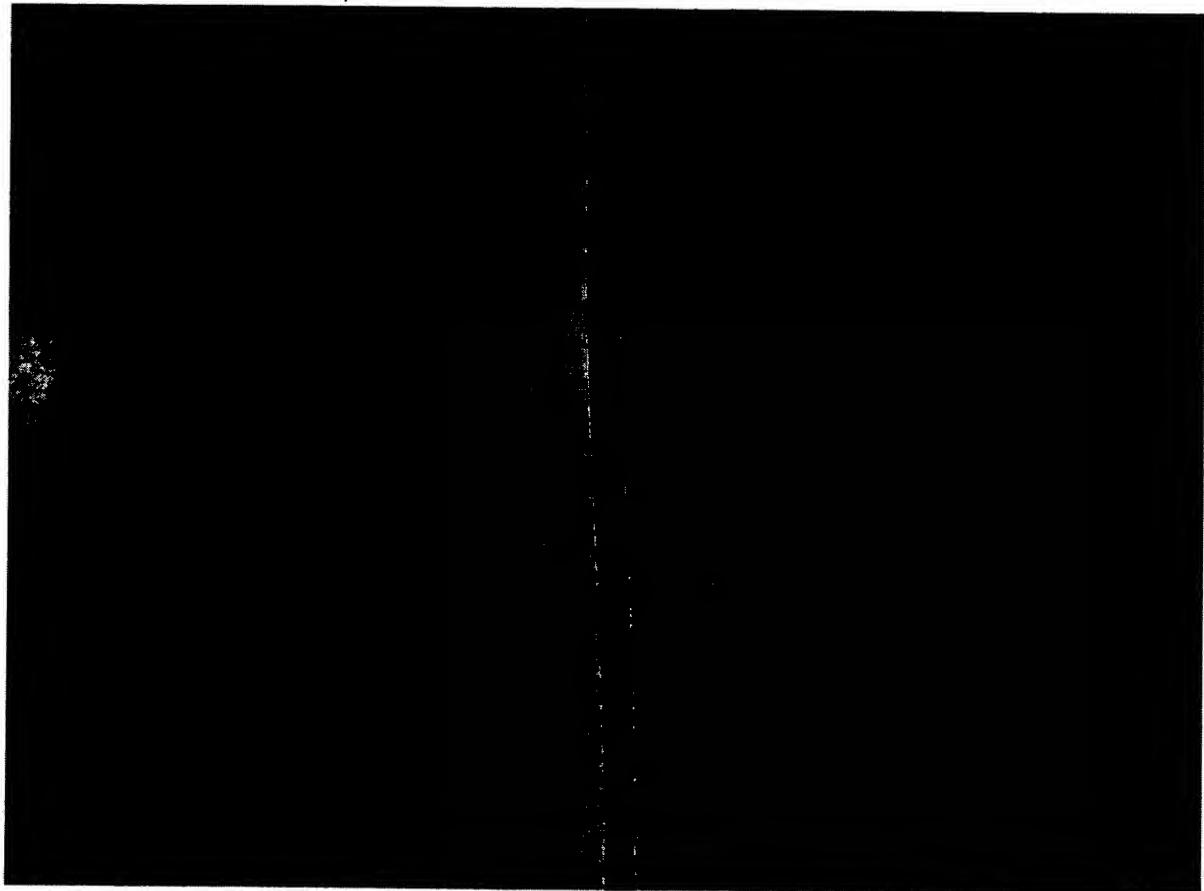


Fig. 12

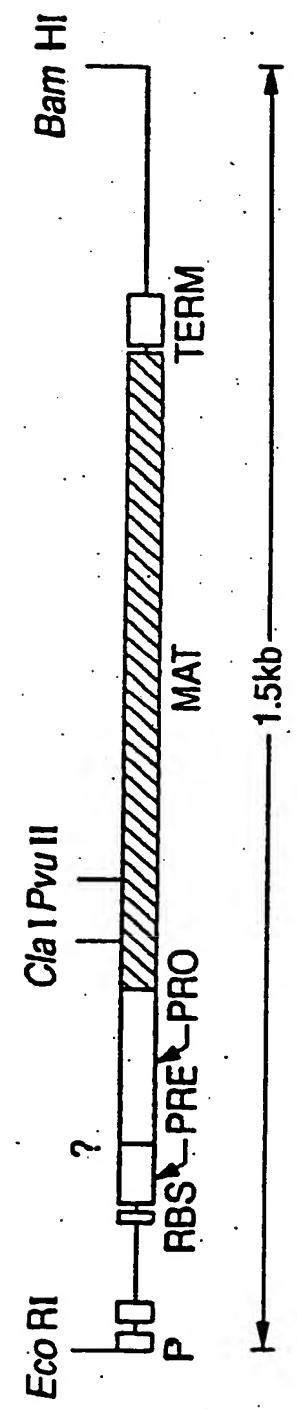


Figure 13.A

FIGURE 13.B1

50	Ala	Gly	Ala	Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp	Asn	Ser	His	Gly	Thr	His	Val	Ala												
549	GCA	GGC	GGG	GCA	GGC	AGC	TCT	CCT	TCT	ATG	GTT	CCT	ATC	GAA	ACA	AAT	CCT	TCA	ATC	CAC	AAC	AAC	TCT	CAC	GGA	ACT	CAC	GTT	GCC						
70	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Le	Gly	Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Ala	Ser	Leu	Tyr	Ala	Val	Ala				
624	GTC	GGT	GCT	CTT	ATC	GGT	GCT	CTT	AAT	ATC	GGT	GTA	ATC	GTC	GGC	CAA	TAC	GGC	ATC	GAG	TGG	GCG	ATC	GCA	AGC	CCA	AGC	GCA	TCA	CCT	TAC	GCT	GTA	AAA	
110	Asp	Ala	Asp	Ala	Asp	Gly	Ser	Gly	Gly	Tyr	Ser	Tyr	Le	Ile	Asn	Gly	Le	Gly	Tyr	Ala	Le	Asn	Asn	Asn	Met										
699	GTT	GTC	GGT	GCT	CTC	GAC	GGT	TCC	GGC	CAA	TAC	GGC	TGG	ATC																					
120	Asp	Val	Le	Asn	Met	Ser	Leu	Gly	Gly	Pro	Ser	Gly	Ser	Ala	Ala	Leu	Ala																		
774	GAC	GTT	ATT	ATC	ATC	ATG	AGC	CTC	GGC	GGG	CCT	TCT	GGT	TCT	GCT	GAT	AAA	GCG	GCA	GTT	GAT	AAA	GCC	GTT	GCA	GCT									
130	Ser	Gly	Val	Val	Ala	Ala	Ala	Gly	Asn	Gly	Thr	Ser	Gly	Ser	Ala	Ala	Val	Asp	Lys	Ala	Val	Asp	Lys	Ala	Val	Ala	Val	Ala	Val	Ala	Val	Ala			
849	TCC	GGC	GTC	GTA	GTC	GTT	GCG	GCA	GCC	GGT	AAC	GAA	GGC	ACT	TCC	GGC	AGC	TCA	AGC	ACA	GTG	GGC	TAC	CCT	GCT	GGT									
150	Ser	Gly	Val	Val	Ala	Ala	Ala	Gly	Asn	Gly	Thr	Ser	Gly	Ser																					
170	Lys	Tyr	Pro	Ser	Val	Le	Ala	Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Pro	Ser	Ser	Val	Gly	Tyr	Pro	Gly	Pro	Gly	Pro	Gly	Pro	Gly		
924	AAA	TAC	CCT	TCT	GTC	ATT	GCA	GTA	GGC	GCT	GCT	GAC	AGC	AGC	AAC	CAA	AGA	GCA	TCT	TTC	TCA	AGC	GTA	GGG	GGT	CCT	GCT	TCA	GGT	GGT	GGT	GGT	GGT	GGT	
180	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Le	Gly	Ser	Thr	Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly	Asn											
999	GAG	CTT	GAT	GTC	ATG	GCA	CCT	GGC	GTA	TCT	ATC	CAA	AGC	AGC	CTT	CCT	GGA	AAC	AAA	TAC	GGA	GGG													
220	Thr	Ser	Met	Ala	Ser	Pro	His	Val	Ala	Gly	Ala	Ala	Ala	Ala	Ala	Ala	Leu	Le	Leu	Ser	Lys	His	Pro	Asn	Tyr	Thr	Asn	Gly	Asn	Asn	Asn	Asn			
1074	ACG	TCA	ATG	GCA	TCT	CCG	CAC	GTT	GGC	GCG	GCT	ATT	CTT	TCT	AAG	CAC	CCG	AAC	TGG	ACA	AAC	GGT	ACT	GGT	ACT										

Figure 13.82

270 Val Gln Ala Ala Gln DC
 275 CAG GCG GCA GCT CAG TAA AACATAAAAACGGCCCTGGCCCCGGTTATTTCCTCTGTTCAATCCGCTC
 280 TERAM

1316 ATATCCGACGGTGGCTCCCTTGAAAATTTACGAGAACGGGGGTGACCCGGCTAAGGCCAAGTCTCAATCGCC

1416 CTCCTCCCTTCCCTTCATGCCCTAACGGTCGGGGTTCCTGATACCGGGAGACGGCATTCGTAATCGGATC

FIGURE 13. B3

CONSERVED RESIDUES IN SUBTILISINS FROM
BACILLUS AMYLOLIQUEFACIENS

1	10	20
A Q S V P . G	A P A . H . . G	
21	30	40
. T G S . V K V A V . D . G	H P	
41	50	60
D L . . . G G A S . V P	Q D	
61	70	80
. N . H G T H V A G T . A A L N N S I G		
81	90	100
V L G V A P S A . L Y A V K V L G A . G		
101	110	120
S G . . S . L . . G . E W A . N		
121	130	140
V . N . S L G . P S . S A . .		
141	150	160
. G V . V V A A . G N . G . . .		
161	170	180
. Y P . . Y A V G A .		
181	190	200
D . . N . . A S P S . . G . . L D . . A		
201	210	220
P G V . . Q S T . P G . . Y . . . N G T		
221	230	240
S M A . P H V A G A A A L . . . K . . .		
241	250	260
W . . . Q . R . . L . N T . . . L G . . .		
261	270	
. . Y G . G L . N . . A A . .		

FIGURE 14

COMPARISON OF SUBTILISIN SEQUENCES FROM:

B. amyloliquefaciens

B. subtilis

B. licheniformis

B. lentus

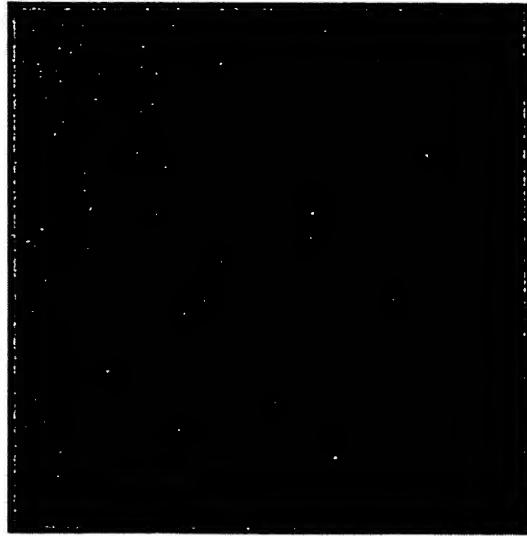
01	10	20	30	40	50	60	70	80	90	100	110	121	130	140	150
	Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S C I D S S H P	Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S C I D S S H P	Q S V P Y G I P P L I K A D K V Q A Q C P K G A N V K V A V I D S C I D S S H P	D L K V A G G A S M V P S E T N P P Q D N N S H G T H V A G T V A A L N N S I G	D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T V A A L N N S I G	D L N V V G G A S F V A G B A Y N * T D G N G H G T H V A G T V A A L D N T T G	D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T V A A L N N S I G	V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D	V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D	L G V A P S V S L Y A V K V L N S S G S G Y S G I V S G I E W A T T N G M D	V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H	V I N M S L G C P S G S A A L K A A V D K A V A S G V V V A A G N E G T S C	V I N M S L G C P T G S T A L K T V V D K A V S S G I V V V A A A G N E G S S C	V I N M S L G C A S G S T A N K Q A V D N A Y A R G V V V A A A G N S G N S C	V A N L S L G S P S P S A T L B Q A V N S A T S R G V L V V A A S G N S G A G S

FIGURE 15.A

Figure 15.B

Initial evaluation results

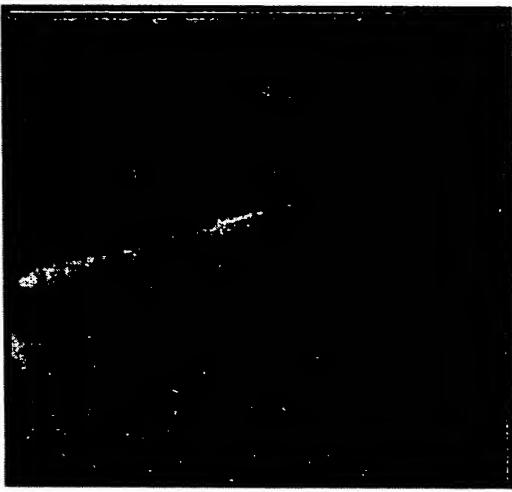
MC-A



MC-3



MC-4

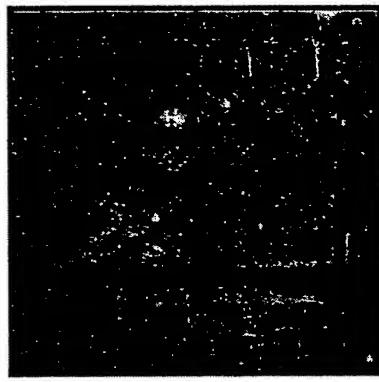


m mbh 2 4 6 8 10 12 P m m mbh 2 4 6 8 10 12 P m

Fig. 16

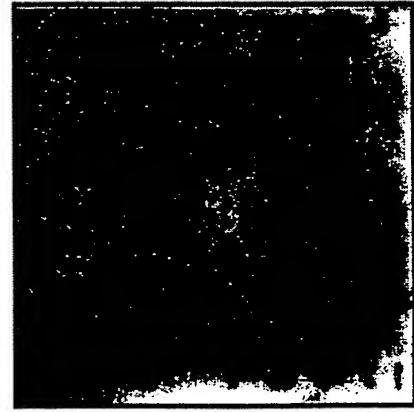
Comparison with Properase

Properase 60°C 30 minutes



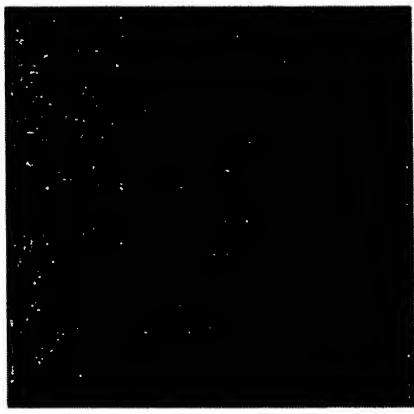
m 2 4 6 8 10 12 P rPrP m

MC-A 50°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

MC-3 50°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

MC-4 50°C 30 minutes



m 2 4 6 8 10 12 P m

Fig. 17

Comparison with Properase

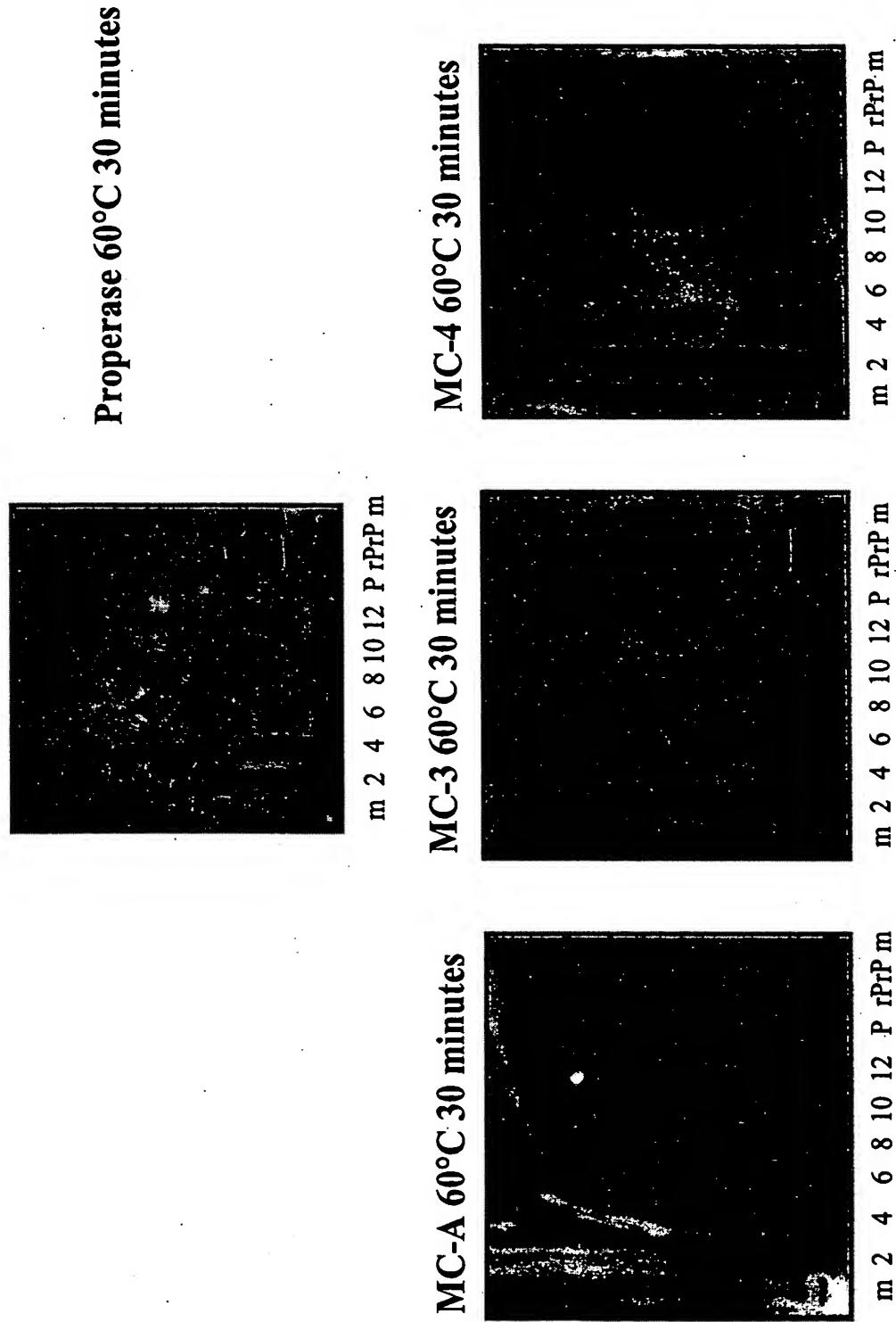


Fig. 18

Temperature profiling with MC-3

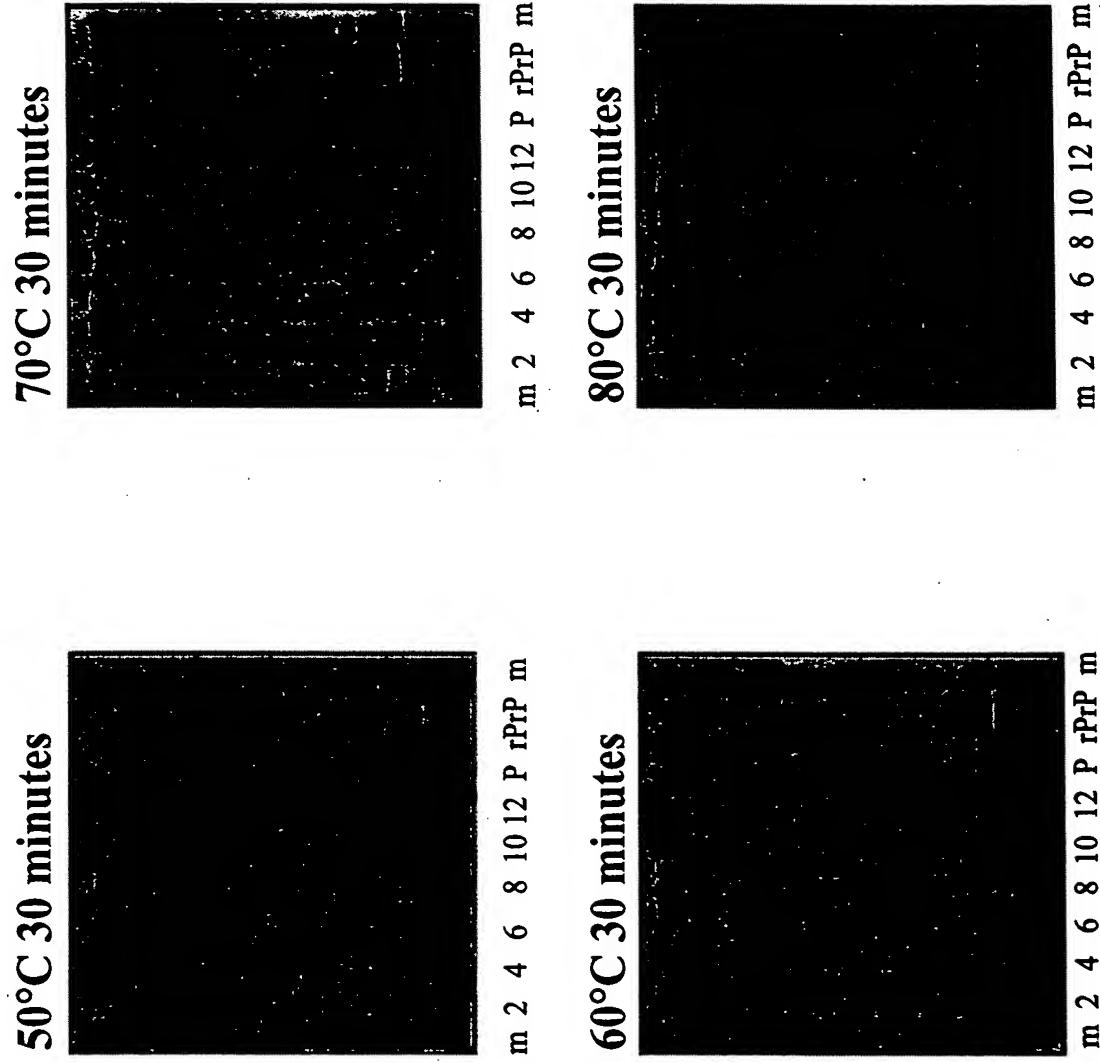


Fig. 19

Detection with PAb2

mbh pH 2-12 digested at 50°C 30 minutes

- Detected with a chemiluminescent detection substrate (Pierce)

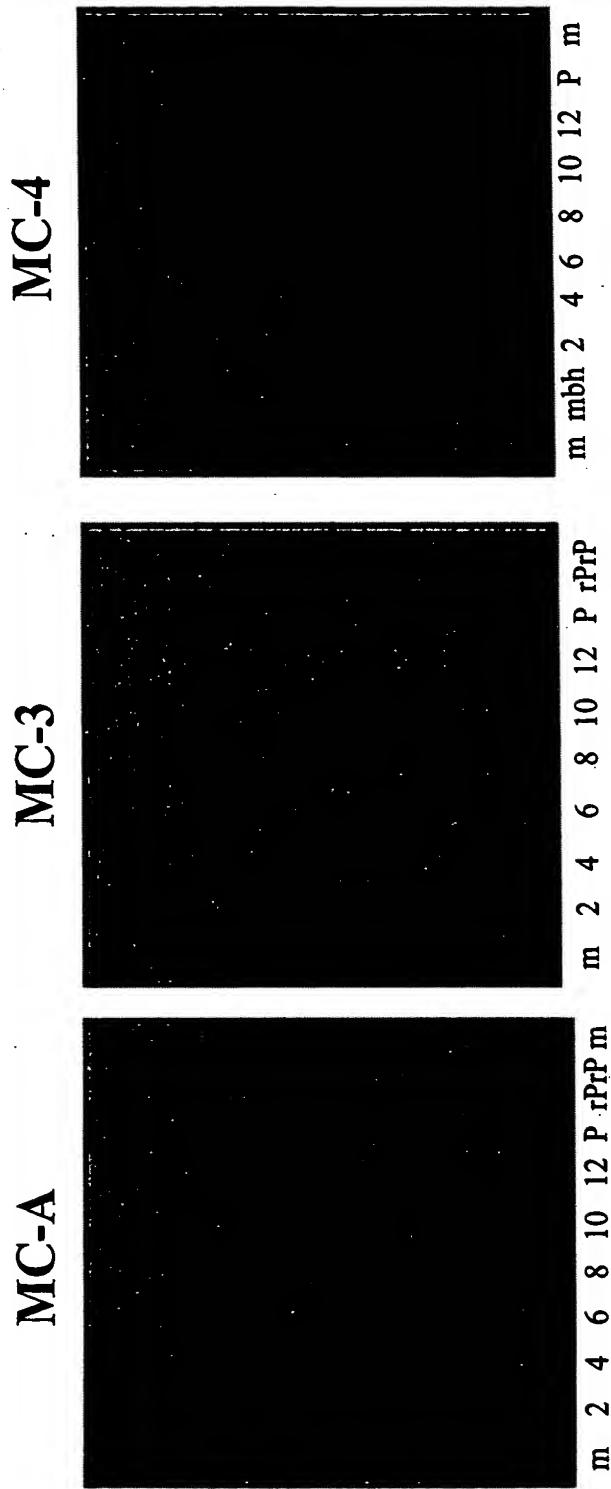


Fig. 20

MC-3 dilutions at pH 10 & pH12

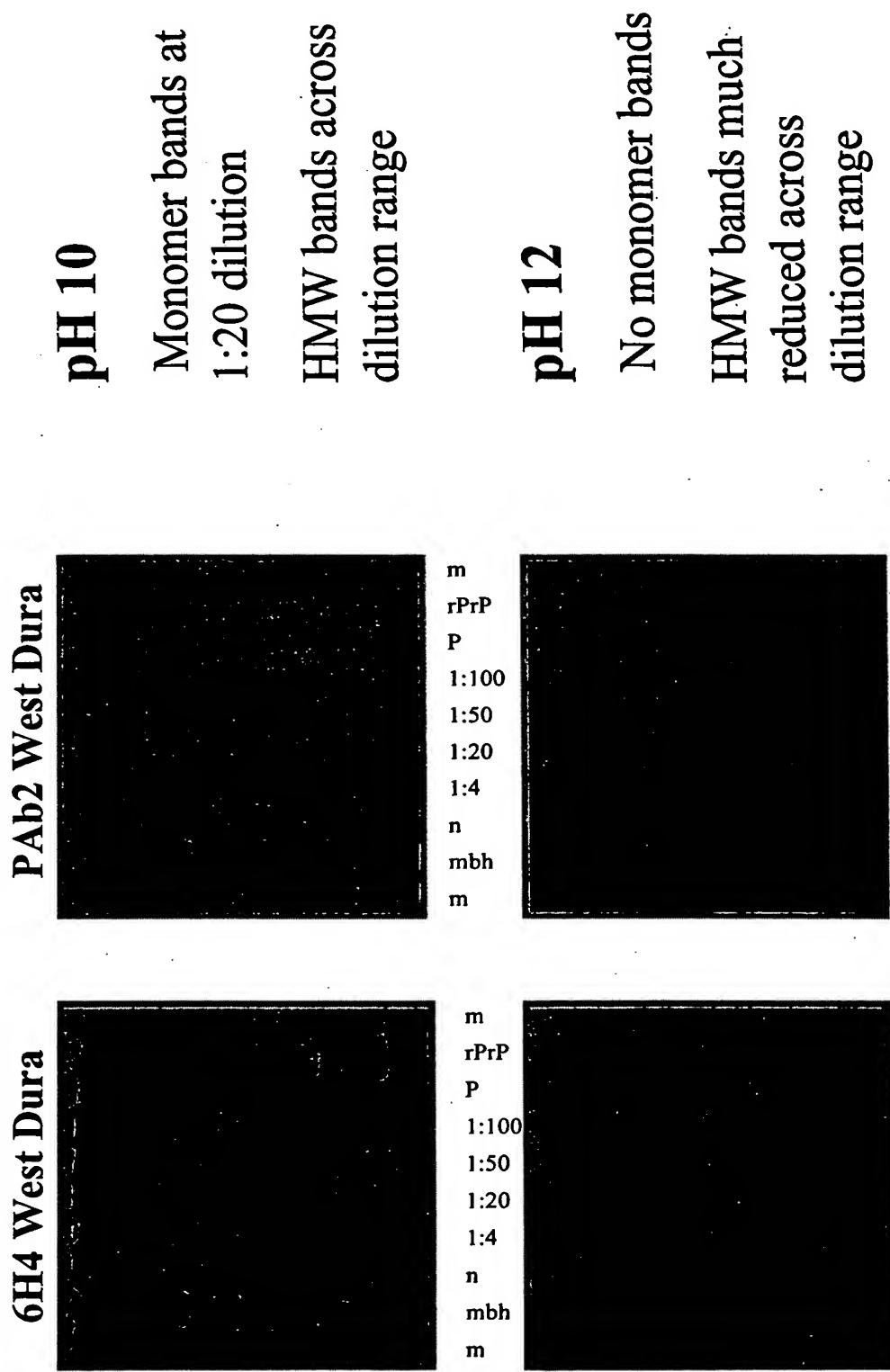
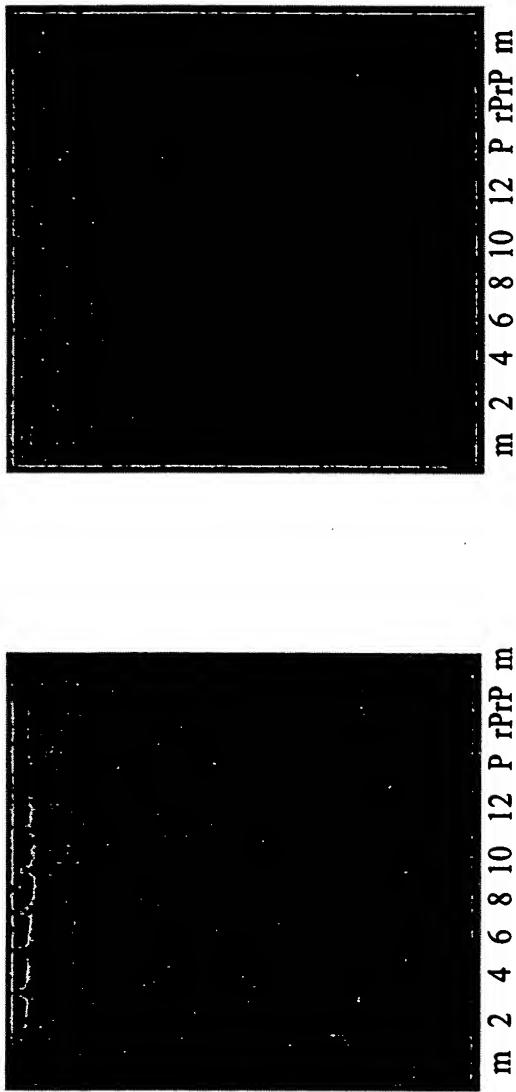


Fig. 21

Comparison with Proteinase K



- Characteristic PrP^{Sc} monomer bands pH 2-10
Incomplete digestion pH12 however no clear monomers
HMW bands present pH 2-12
The new proteases are better at removing both the monomer and HMW bands than Proteinase K

Fig. 22